



Effects of Nanobubble Water on Cultivation of Lactobacillus acidophilus 1028 and Community of Gut Microbiota in Mice

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論文の要旨

Abstract of thesis

Water is the most important factor for life, and its changes in properties can affect the composition of gut microbiota. Nanobubble water (NBW) is a kind of water with numerous nanobubbles (NBs) inside, which has been applied in many fields due to its unique properties. Recently, many works reported the promotion effects of NBW on plants, animals and cells. However, little information is available on the effects of NBW on microorganisms, especially on the gut microbes. This dissertation investigated the potential effects of NBW on host health and disease through the modulation of gut microbes. This dissertation is divided into 5 parts.

In chapter 1, the author introduced the close relationship between gut microbiota and the host from the role of gut microbes in the maintenance of host health and the development of diseases. Especially, in this chapter, the author conducted literature reviews on two important properties of NBW and application fields.

In chapter 2, the author firstly studied the properties and stability of NBW. Then, with *Lactobacillus acidophilus* (LA)1028 being used as the model strain, the in vitro effects of four kinds of NBW prepared with different gases on the strain growth performance were explored. Results indicated that nitrogen NBW (N₂-NBW) had the highest absolute value of zeta potential, NB density and water mobility (-25.3 ± 5.43 mV, $(5.73 \pm 1.0) \times 10^7$ particles/mL and (3200 ± 139.6) ms, respectively), while the lowest was detected in carbon dioxides NBW (CO₂-NBW) (-6.96 ± 2.36 mV, $(3.39 \pm 1.73) \times 10^7$ particles/mL and (2764.6 ± 40.1) ms, respectively). Besides, the NBs could stably exist in the N₂-NBW and hydrogen NBW (H₂-NBW) during 30 days' storage. Moreover, the author found that except CO₂-NBW, all the other NBW showed some promotion effect on the growth of the strain at lag and logarithmic phases. Among them, N₂-NBW demonstrated the best performance, achieving the highest increase ratio of 51.1% after 6 h cultivation. The preliminary analysis of the mechanisms suggested that these effects were related to the properties (zeta potential and density) of NBs, which might affect the transport of substances. These results suggest that NBW has the potential for

promoting the production efficiency of probiotics via fermentation.

In chapter 3, the author studied the effects of NBW on gut microbiota of the host. The mice were fed with the standard diet (SD) and were separately supplemented with N₂-NBW (SD-N₂ group), H₂-NBW (SD-H₂ group) and deionized water (SD-C group) for five weeks. At the end of the experiment, the author analyzed the composition of fecal microbiota using the 16S rRNA gene sequencing. Results indicated that the species diversity in the SD-N₂ group was significantly increased than that in the SD-C group, while the SD-H₂ group showed no difference with the SD-C group. Compared with the SD-C group, the ratio of *Firmicutes* to *Bacteroidetes* in the SD-N₂ group was significantly increased. That's mainly due to the relative abundance of S24-7 was significantly reduced at the family level and the relative abundance of *Clostridium* and *Coprococcus* was significantly increased at the genus level. In the SD-H₂ group, the relative abundance of *Mucispirillum* and *Helicobacter* was found significantly decreased than those in the SD-C group. Overall, supplementation with NBW to mice could alter the composition of gut microbiota in mice.

In chapter 4, the author investigated the effects of NBW on the process of obesity in mice under high-fat diet (HFD). In the experimental groups, the author supplemented the HFD and N₂-NBW (HFD-N₂ group) or H₂-NBW (HFD-H₂ group) to mice, while the mice fed with SD (SD-C group) or HFD (HFD-C group) were supplemented with deionized water were used as control groups. After ten weeks, the author found that the concentration of total cholesterol, alanine aminotransferase and lipopolysaccharide in the mice serum of the HFD-N₂ group were significantly lower than those in the HFD-C group. The results from 16S rRNA gene sequencing revealed that supplementation with N₂-NBW to mice significantly inhibited the ratio of *Firmicutes/Bacteroidetes* increase. The results from the Spearman's correlation analysis indicated that the alteration of gut microbiota had a close relationship with the changes of obesity-associated markers, indicating that supplementation with N₂-NBW could potentially alleviate the process of obesity in HFD-fed mice through modulation of gut microbiota.

In chapter 5, the author summarized the major conclusions from the whole study and prospected future researches on the mechanisms involved in the NBW effects on the strain and the application of NBW to other chronic diseases.

審査の要旨

Abstract of assessment result

In this study, the author investigated the properties of NBW and the stability of NBs, demonstrating the promotion effects of NBW on the growth of strain LA 1028 and the modulation effects of NBW on the composition of gut microbiota of the host. (1) The medium with and without NBW addition was applied to explore its effects on the growth and metabolism of *Lactobacillus acidophilus* 1028 (LA1028) through evaluating the strain growth, lactic acid production and glucose consumption. Results show that the addition of NBW in the medium promoted the growth and lactic acid production of LA 1028 at lag and logarithmic phases. In addition, supplementation with nitrogen (N₂) and hydrogen (H₂) NBW in addition to deionized water to mice under standard diet for five weeks affected the composition of gut microbiota in mice. 16S rRNA gene sequencing analysis indicated that the N₂-NBW could increase the species diversity and alter the community structure of fecal microbiota in mice. Furthermore, being supplemented with NBW under high-fat diet (HFD) for ten weeks, the author found that supplementation with N₂-NBW to the HFD fed mice could effectively retard the body weight increase in mice. The effects of NBW on the alleviation of obesity were mainly achieved by altering the composition of gut microbiota to impact the energy intake, lipid metabolism and LPS concentration in mice. This study provided a new perspective of NBW on the production of probiotic and in the alleviation of obesity, which can be supplied a factual basis for the application of NBW in the food and medicine fields.

The final examination committee conducted a meeting as a final examination on 17th January, 2020. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

Therefore, the final examination committee approved that the applicant is qualified to be awarded the degree of Doctor of Philosophy in Environmental Studies.